6

Auxiliary Techniques

The limitations of the structural information in the normal mass spectrum can be partly offset by special mass-spectral techniques. Although a complete description of these is beyond the scope of this book, you should have a basic familiarity with their capabilities.

6.1 Soft ionization methods

Low-energy electron ejection. Lowering the energy of the bombarding electrons (e.g., to 15 eV) will also lower the average (but *not the minimum*) internal energy of the molecular ions. Such low-energy $M^{+\cdot}$ ions can also be produced by field ionization, charge exchange, or photoionization (Section 7.4). For structure elucidation it is sometimes useful to eliminate higher-energy reactions that give secondary product ions that are much less representative of the original structure. However, lower electron energies increase the relative abundance of primary rearrangement reactions (Section 7.2). Further, lowering the electron energy decreases the *absolute* abundance of all ions; although the *relative* abundance of the molecular ion increases, it nevertheless is more difficult to detect at lower electron energies (unless it has been obscured by fragment ions from a higher-molecular-weight impurity). Lowering the molecule's energy (such as with supersonic expansion, Amirav 1991) before ionization does produce $M^{+\cdot}$ of even lower internal energy, increasing $[M^{+\cdot}]$ (Section 7.4).

EE^+ ***molecular species from ion-molecule reactions.*** The most common method of producing a stable ion containing the sample molecule is to react it with an ionized EE^+ species. An early example was the use of high sample pressures under EI conditions to produce MH^+ ions from compounds not yielding $M^{+\cdot}$ (McLafferty 1957b). Chemical ionization (CI; see Munson and Field 1966, Harrison 1992) is very useful, in that most (although not all) molecules that do not yield molecular ions by EI can produce CI ions indicative of the molecular weight. CI conditions produce abundant thermal electrons, giving highly efficient electron capture for negative ion formation from electronegative molecules (Figure 1.2; Budzikiewicz 1981, 1983b; Bowie 1984, 1989; Hites 1988). Recently a wide variety of powerful methods for the ionization of large molecules (even of molecular weight $>10^6$ have been developed; most yield such EE^+ molecular species (Section 6.2).

Chemical ionization. For CI the reagent gas R is introduced into the ion source ($\sim$0.5 torr, $\sim$70 Pa) at a concentration in large excess ($\sim 10^4:1$) to that of the sample, and is ionized by electron ($\sim$500 V) bombardment or electric discharge (Hunt 1975). The $R^{+\cdot}$ ions initially formed can react further with other R molecules to form reactive ion species that then attack the sample molecule M:

$$R + e^- \longrightarrow R^{+\cdot} + 2e^- \tag{6.1}$$

$$R^{+\cdot} + R \longrightarrow RH^+ + (R-H)\cdot, \text{ or } (R-H)^+ + RH\cdot \tag{6.2}$$

$$RH^+ + M \longrightarrow R + MH^+ \quad \text{(protonation)} \tag{6.3}$$

$$(R-H)^+ + M \longrightarrow (R-2H) + MH^+ \quad \text{(protonation)} \tag{6.4}$$

$$(R-H)^+ + M \longrightarrow R + (M-H)^+ \quad \text{(hydride abstraction)} \tag{6.5}$$

$$R^{+\cdot} + M \longrightarrow R + M^{+\cdot} \quad \text{(charge exchange)} \tag{6.6}$$

Among this wide variety of possible ionization reactions, the most common are: protonation (Equations 6.3 and 6.4), which is favored for sample molecules of proton affinity higher than that of the reagent; hydride abstraction (Equation 6.5), which is common for lower-proton-affinity molecules, such as alkanes; and charge exchange (6.6), which is favored for reagents of high ionization energy, such as helium. The internal energy of the resulting ionized M species depends on the reagent chosen and the ion source temperature and pressure (number of thermalizing collisions). For example, Figure 6.1 (Fjeldsted *et al.* 1990) shows the CI spectra of lavanduyl acetate (PA = $\sim$8.7 eV) using CH_4 (PA = 5.7 eV), isobutane (reagent $C_4H_9^+$, PA of *iso*-C_4H_8 = 8.5 eV), and NH_3 (PA = 9.0 eV). Further dissociation is nearly complete for the highly exothermic CH_5^+ reaction, and minor for the NH_4^+ reaction. Such unimolecular fragmentations obey the mechanisms outlined in Chapters 4 and 8; the major *m/z* 137 product ion is formed by CH_3COOH loss, consistent with Field's Rule: $PA(CH_3COOH) = 8.2$ eV, $PA(C_{10}H_{16}) = \sim 8.7$ eV. The *m/z* 154 product in the NH_3/CI spectrum corresponds to CH_3COOH loss from $(M + NH_4)^+$. Thus CI spectra can provide structural as well as molecular weight information. Similar CI spectra can

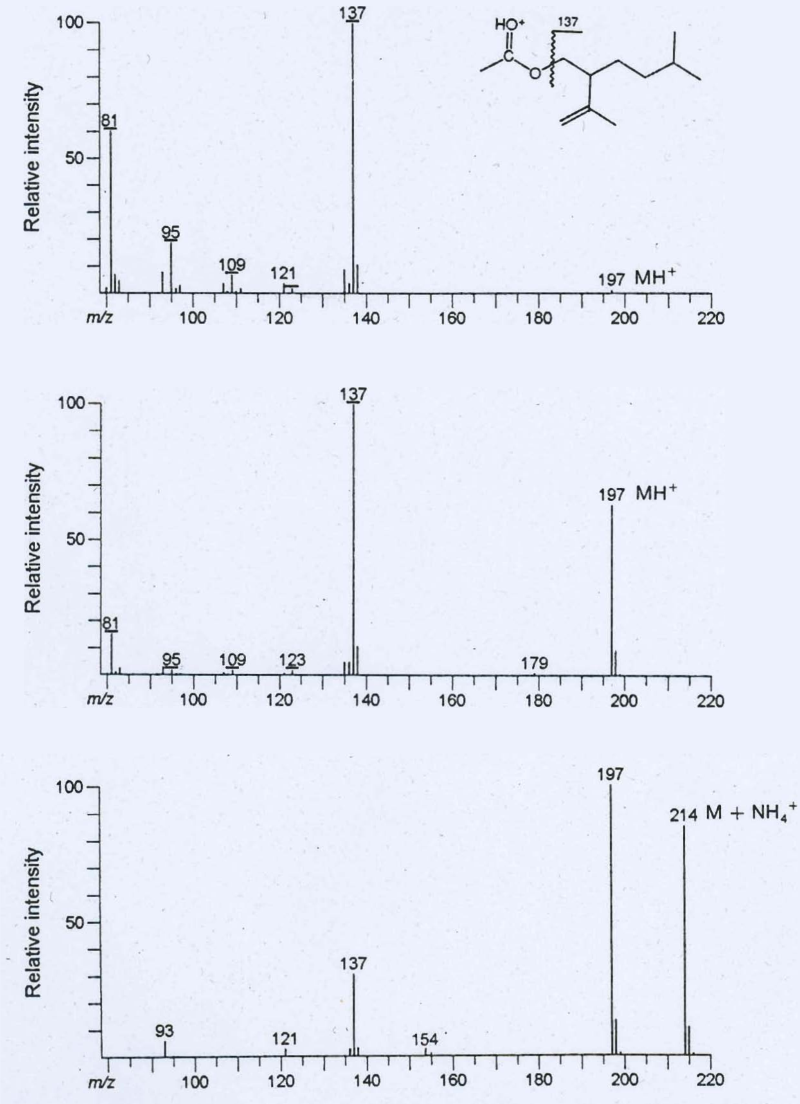

Figure 6.1. The CI mass spectra of lavanduyl acetate (MW 196), using CH_4, iso-C_4H_{10}, and NH_3 as reagent gases.

be generated at much lower pressures by using long-lived trapped ions in the ion cyclotron resonance (ICR; see Lehman and Bursey 1976, and Bowers 1979, 1984) or ion trap (IT; see Cooks *et al.* 1991) instruments. For definitions of PA and gas-phase acidity and basicity, see Bursey 1990.

6.2 Ionization of large molecules

For conventional direct-probe introduction, a sample must have sufficient vapor pressure and thermal stability to be vaporized in the ion source. This severely limits the application of mass spectrometry to important samples such as biological macromolecules, industrial polymers, and petroleum residues. Intensive recent research progress has resulted in highly useful methods. The most widely used are described briefly here; many recent references (Section 6.8) give extensive details.

Fast-atom bombardment (FAB). Introduced by Barber *et al.* (1982), FAB is particularly suitable for polar molecules, providing ionized molecular species for molecular weights up to ~20 kDa (Williams *et al.* 1987; Tower 1989). A solution of the sample in a low-volatility matrix such as glycerol is bombarded with fast, heavy atoms (e.g., Xe; ions such as Cs^+ give similar results) in the ion source, producing the continuous ion beam necessary for scanning instruments. The method is very convenient for appropriate samples, but matrix reactions produce background ions at nearly every mass value.

Plasma desorption (PD) and laser desorption (LD). These pulsed ionization techniques are particularly suitable for the time-of-flight instrument, which has the additional advantage of unlimited mass range. TOF resolving power of 500–1,000 has been a disadvantage, but recent improvements are promising (Benninghoven 1989; Karas *et al.* 1989; Hillenkamp *et al.* 1991). PD, pioneered by Macfarlane (1976), first showed that mass spectra could be obtained routinely from large molecules. Fission of ^{252}Cf produces two 100-MeV products that simultaneously ionize the thin-film samples and start the TOF clock, but produce little ion fragmentation. Ionization of molecules as large as 50 kDa with mass measuring errors of ±0.1% have been reported.

Pioneering laser desorption (LD) capabilities (Posthumus *et al.* 1978) have been greatly extended (Karas and Hillenkamp 1988) by dissolving the sample in a solid matrix that is a highly efficient absorber of the laser radiation, so as to ionize molecules as large as 300 kDa (Hillenkamp *et al.* 1991). The absence of matrix effects and mass measuring errors of ±0.01%–0.1% make LD a particularly promising method for protein mixtures (Chait *et al.* 1990).

Electrospray ionization (ESI). Pioneered by Dole (Dole *et al.* 1971), for ESI a solution of the sample is sprayed at atmospheric pressure through a several-kilovolt potential difference toward the differentially pumped entrance to the mass spectrometer (Smith *et al.* 1992). The resulting droplets are electrostatically charged; as the solvent evaporates, electrostatic repulsion produces smaller and smaller droplets, until the macromolecule is expelled "saturated" with charges (Fenn *et al.* 1989). Thus a protein can bear a proton for every 5–17 amino acids, yielding peaks at m/z 600–2000 even for 200 kDa proteins (Feng *et* *al.* 1991). Similarly, polynucleotides can yield negative ions of such *m/z* values by losing a proportionate number of protons. This drastically reduced upper limit for *m/z* measurement makes ESI amenable to most types of mass spectrometers. ESI mass spectra have been measured for molecules as large as 5×10^6 Da (Nohmi and Fenn 1992), and structural information has been obtained from albumin (66 kDa) by tandem mass spectrometry (Loo *et al.* 1991). Using Fourier-transform (ICR) mass spectrometry (Section 1.3), ESI spectra of myoglobin (17 kDa) show 900,000 resolving power and <0.001 Da mass-measuring errors (Beu *et al.* 1993).

Pyrolysis. Useful structural information about complex mixtures of high molecular weight, such as bacteria, plant specimens, coal, high polymers, and sewage sludge, can be obtained from the mass spectra of their thermal decomposition products (Meuzelaar *et al.* 1980, 1989; Schulten 1986; Ballistreri *et al.* 1991). To minimize secondary reactions, small samples (10^{-4} to 10^{-7} g) are preferable, as are reproducible pyrolysis conditions, such as rapid RF heating of a wire to its Curie temperature. Laser, spark, and other flash-heating methods have also been used. Of particular interest is an automated system for continuous particle beam sample intoduction with Curie-point pyrolysis in the MS vacuum chamber. This system can distinguish different strains of bacteria, reporting results every 15 s (Snyder and Harden 1990).

6.3 Exact mass measurements (high resolution)

Measurement of the mass of an ion with sufficient accuracy provides an unequivocal identification of its elemental (and isotopic) composition. This technique is often referred to as "high resolution" mass spectrometry, since double-focusing (Figure 6.2) or FT/ICR (Section 1.3) instruments are usually used. However, if interfering masses requiring high resolution are absent, sufficiently accurate mass measurements (~20 ppm) can be achieved for low-mass ions with single focusing or quadrupole instruments of 1,000–2,000 resolution.

Elements can be identified in this way because monoisotopic atomic weights are not exact whole numbers. When we take as standard the mass of ^{12}C as 12.00000000 (see Table A.1), then *m* for $^{1}H = 1.007825035$, for $^{14}N = 14.00307400$, and for $^{16}O = 15.99491463$ (the least significant figure, 10^{-8} daltons, represents 9.3 eV of energy). Every isotope has a unique, characteristic "mass defect"; so the mass of the ion, which shows the total mass defect, identifies its isotopic and elemental composition. For example, an ion with a mass of 43.0184 must be $C_2H_3O^+$; it cannot be $C_3H_7{}^+$ ($m = 43.0547$), $C_2H_5N^{+\cdot}$ ($m = 43.0421$), $CH_3N_2{}^+$ ($m = 43.0296$), $CHNO^{+\cdot}$ ($m = 43.0058$), or C_2F^+ ($m = 42.9984$). To distinguish these compositions requires an accuracy in measuring mass of 130 ppm.

The usefulness of elemental composition information increases exponentially with increasing mass, as does the required mass-measuring accuracy. Figure